

## (Measurement of Cell Survival Rate)

**[0174]** After standing for 2 hrs, the aforementioned 15 mL tube was removed from the incubator, the cell aggregates were well dispersed by blending with inversion. 0.75 mL of the culture medium was collected and 0.75 mL of ATP reagent (CellTiter-Glo (registered trade mark) Luminescent Cell Viability Assay, manufactured by Promega) was added and the mixture was well stirred with Pipetman. After allowing to stand at room temperature for 10 min, 100  $\mu$ L of each was dispensed into a white 96-well plate, the luminescence intensity (RLU value) was measured with Enspire (manufactured by Perkin Elmer), and the number of viable cells was measured by subtracting the luminescence value of the medium alone.

**[0175]** The relative value when the RLU value (ATP measurement, luminescence intensity) of the suspension before division was 100% was taken as the cell viability.

## (Evaluation of Effect of Backflow Washing of Mesh Structure)

**[0176]** The graphs of FIGS. 8(a) and (b) show the results of Experimental Example in which the division was continued without performing the backflow washing of the mesh structure of the above-mentioned (i). The graph of FIG. 8(a) shows the results relating to a suspension having a cell concentration of  $3.0 \times 10^5$  cells/mL, and the graph of FIG. 8(b) shows the results relating to a suspension having a cell concentration of  $6.0 \times 10^5$  cells/mL.

**[0177]** The graphs of FIGS. 8(c) and (d) show the results of Experimental Example in which the division was continued while performing the backflow washing of the mesh structure of the above-mentioned (i). The graph of FIG. 8(c) shows the results relating to a suspension having a cell concentration of  $3.0 \times 10^5$  cells/mL, and the graph of FIG. 8(d) shows the results relating to a suspension having a cell concentration of  $6.0 \times 10^5$  cells/mL.

**[0178]** As shown in the graphs of FIG. 8(a) and (b), when the backflow washing of the mesh structure was not performed, cell viability decreased as the amount of the dividing treatment increased at both cell densities. In contrast, as shown in the graphs of FIG. 8(c) and (d), when the backflow washing of the mesh structure was performed, a decrease in the cell survival rate was sufficiently suppressed at a cell density of  $3 \times 10^5$  cells/mL, and a decrease in the cell survival rate was suppressed even at a cell density of  $6 \times 10^5$  cells/mL as compared to the results shown in the graph of FIG. 8(b).

**[0179]** From the above, it was clarified that in the division of the cell aggregate using the mesh structure, the periodic backflow washing of the mesh structure is very effective in suppressing a decrease in the survival rate of the cell aggregate after the division. In addition, it is considered that the decrease in cell survival rate can be suppressed by increasing the frequency of backflow washing of the mesh structure (i.e., at time point when a predetermined number of cell aggregates have passed through a unit area of the mesh structure) since more cell aggregates pass through the mesh structure when the cell density is higher.

[Experimental Example 3] Test on the Relationship between the Cross-Sectional Shape of the Beam Part and the Survival Rate of the Cell Aggregate

**[0180]** Human pluripotent stem cells (hiPS cells) were suspension cultured to form cell aggregates, and the cell aggregates were divided by four types of devices having

different cross-sectional shapes of the beam part, and a test was conducted to confirm the division performance by the cross-sectional shape of the beam part by observing the survival rate of the cell aggregate after each division for each volume.

(Three-Dimensional Culture of hiPS Cell before Division)

**[0181]** medium 1:

**[0182]** Liquid medium composition prepared by injecting 0.016% (w/v) deacylated gellan gum (KELCOGEL CG-LA, manufactured by Saneigen FFI) using FCEM-series Preparation Kit (manufactured by Nissan Chemical Corporation) to mTeSR1 medium (manufactured by STEMCELL Technologies) containing 10  $\mu$ M Y-27632 (manufactured by FUJIFILM Wako Pure Chemical Corporation) according to the mixing method described in patent document 2.

**[0183]** medium 2:

**[0184]** Liquid medium composition prepared by injecting 0.016% (w/v) deacylated gellan gum to mTeSR1 medium according to the mixing method described in patent document 2.

**[0185]** The hiPS cell line 253G1 (distributed from RIKEN) was maintenance cultured in a CO<sub>2</sub> incubator (37° C., 5% CO<sub>2</sub>) in a static state using a variable volume 200 mL culture bag (manufactured by Nipro), medium 1 and medium 2.

**[0186]** The cells were seeded in medium 1 on day 0 of culture, medium 2 was added every 1 to 3 days, and this was continued for 6 to 8 days to form a cell aggregate. On the final day, the cell aggregate was collected using MACS (registered trade mark) Smart Stratifiers (70  $\mu$ m, manufactured by MACS), suspended in medium 1, and then passed through a device according to the device of the present invention, and the divided cell aggregates were seeded (day 0). This was repeated to carry out maintenance culture of the cells.

## (Specifications of the Mesh Structure of the Device)

**[0187]** The instruments used in the following cases were sterilized with ethanol for disinfection.

**[0188]** As an example product of the device, a porous film having the following shape was produced.

**[0189]** (a) The shape of the opening is the regular hexagon shown in FIG. 1(a), and the cross-sectional shape of the beam part is the rectangle shown in FIG. 1(c).

**[0190]** (b) The shape of the opening is the square shown in FIG. 3(a), and the cross-sectional shape of the beam part is the rectangle shown in FIG. 1(c).

**[0191]** (c) The shape of the opening is the regular hexagon shown in FIG. 1(a), and the cross-sectional shape of the beam part is the shape (having a circular arc and a chord) with rounded corners on the inlet side shown in FIG. 3(e)).

**[0192]** (d) The shape of the opening is the square shown in FIG. 3(a), and the cross-sectional shape of the beam part is the shape (having a circular arc and a chord) with rounded corners on the inlet side shown in FIG. 3(e)).

**[0193]** The material of the film body is nickel, the thickness of the film body (thickness t1 in FIG. 1(c), (e)) is 20  $\mu$ m, and the wire diameter (width of the beam part) is 50  $\mu$ m. The pore size of each through-hole (the distance between two parallel sides facing each other among the six sides of the regular hexagon which is the shape of the opening or the distance between the two sides of a square, which is the shape of the opening) is 60  $\mu$ m.